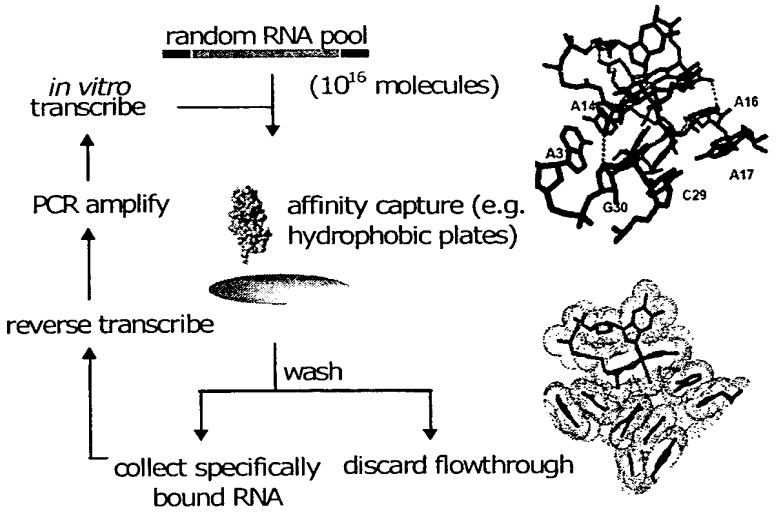

random RNA pool
in vitro transcribe
(10^16 molecules)
A14
A16
A3
A17
C29
G30
PCR amplify
affinity capture (e.g. hydrophobic plates)
reverse transcribe
wash
collect specifically bound RNA
discard flowthrough

Figure 1

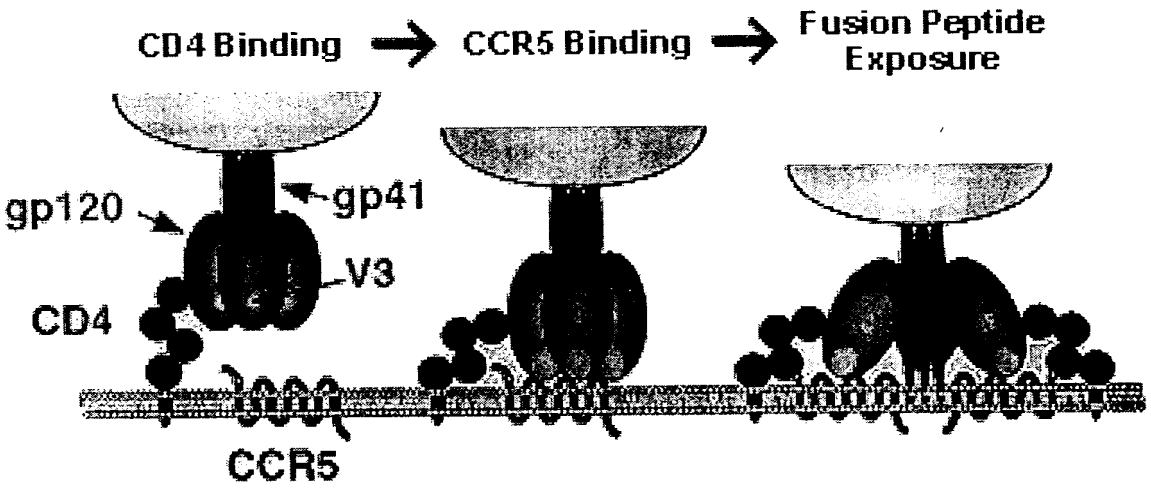
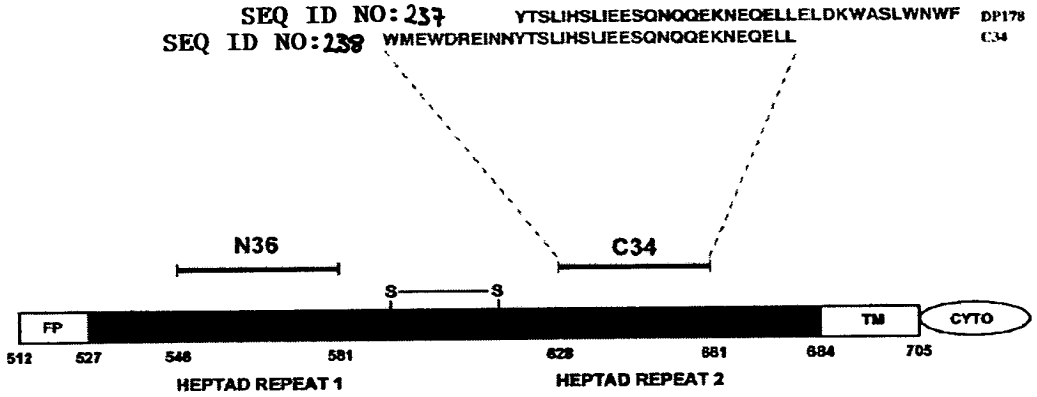
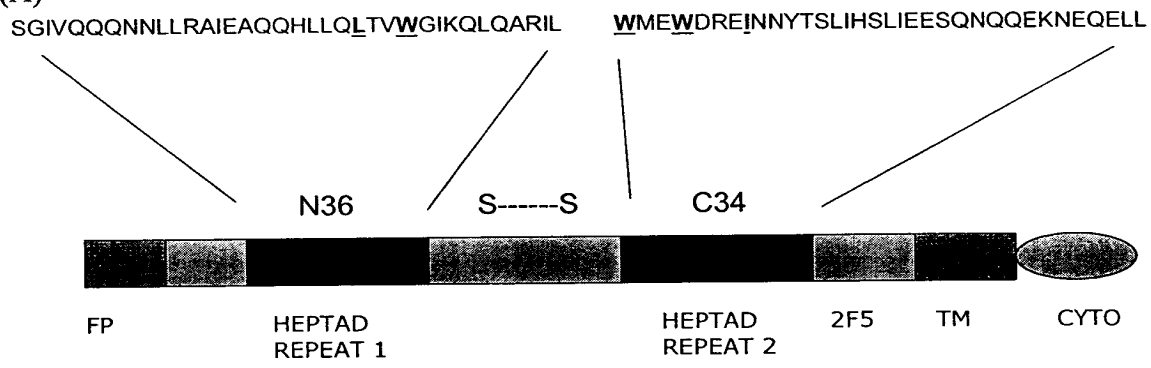

CD4 Binding
CCR5 Binding
Fusion Peptide Exposure
gp120
gp41
V3
CD4
CCR5

Figure 2


SEQ ID NO:237 YTSLIHSLIEESQNQQEKNEQELLELDKWASLWNWF DP178
SEQ ID NO:238 WMEWDREINNYTSLIHSLIEESQNQQEKNEQELL C34
N36
C34
S
S
FP
TM
CYTO
512
527
546
581
628
681
684
705
HEPTAD REPEAT 1
HEPTAD REPEAT 2

(A)


SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARIL
WMEWDREINNYTSLIHSLIEESQNQQEKNEQELL
N36
S------S
C34
FP
HEPTAD REPEAT 1
HEPTAD REPEAT 2
2F5
TM
CYTO

(B)

Figure 3

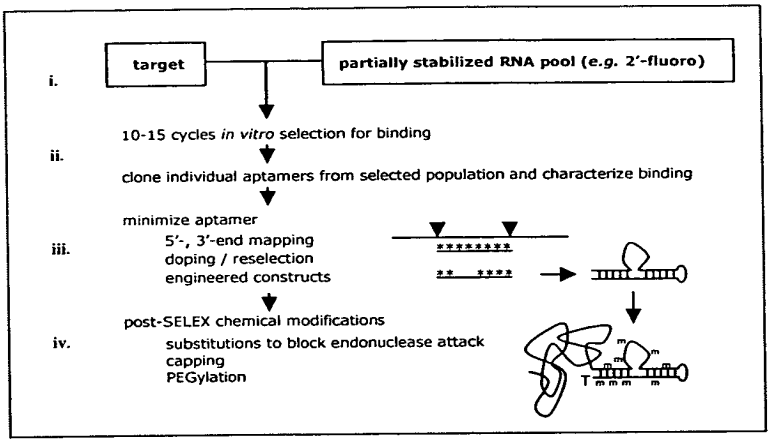

i.
target
partially stabilized RNA pool (e.g. 2'-fluoro)
ii.
10-15 cycles in vitro selection for binding
clone individual aptamers from selected population and characterize binding
iii.
minimize aptamer
5'-, 3'-end mapping
doping / reselection
engineered constructs
iv.
post-SELEX chemical modifications
substitutions to block endonuclease attack
capping
PEGylation

Figure 4

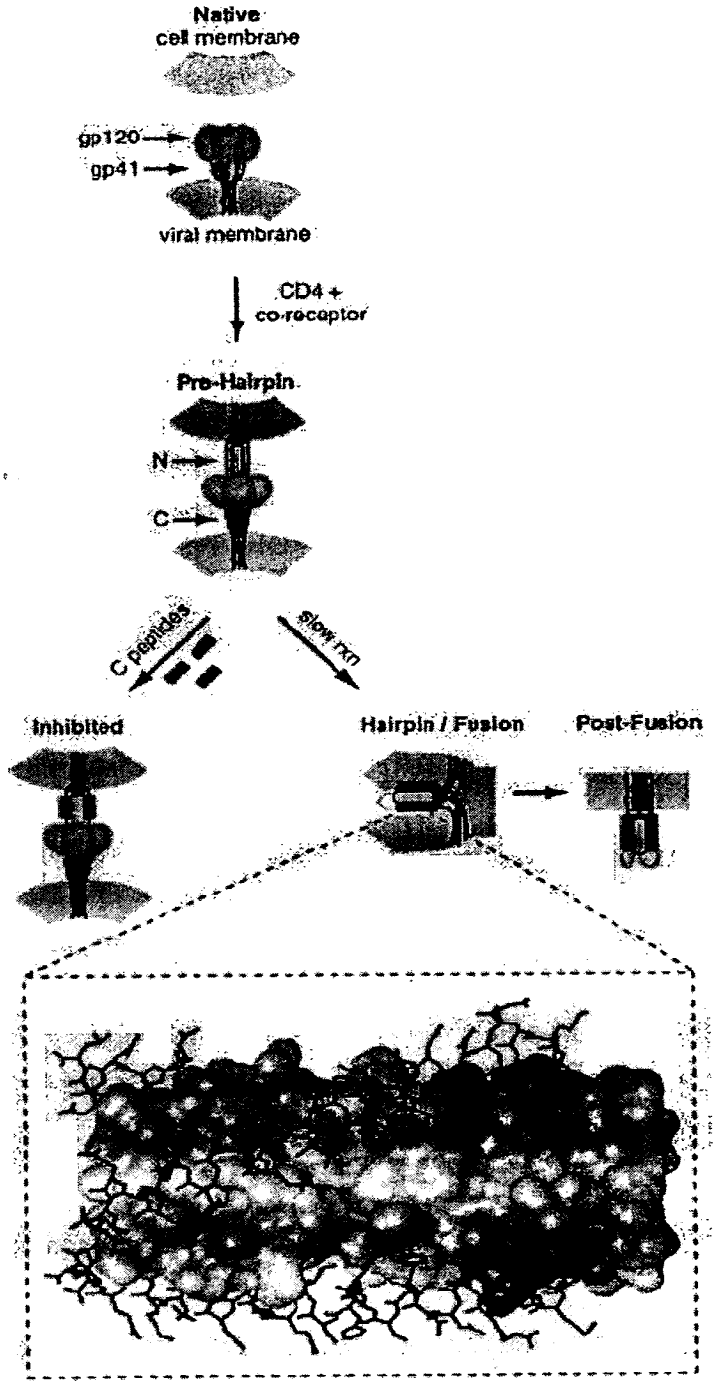
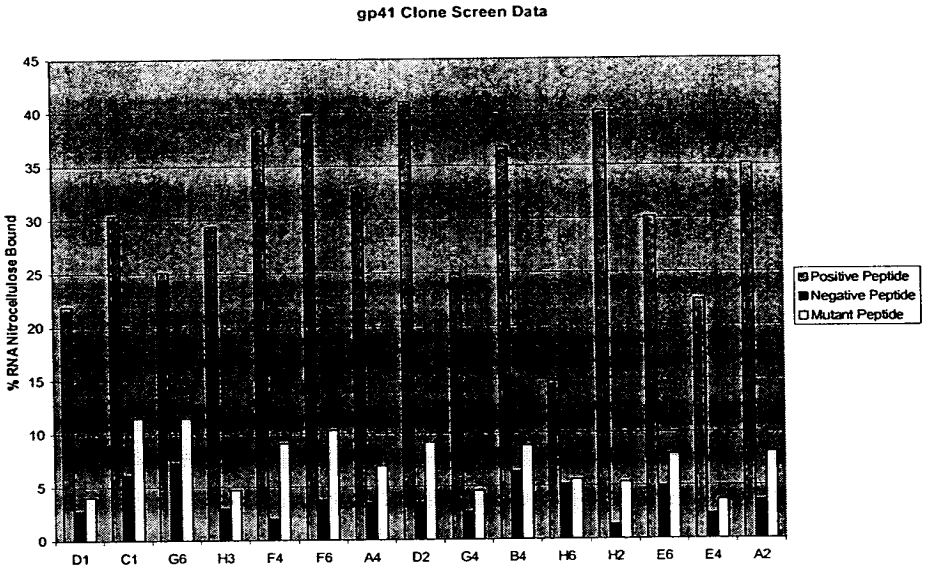

Native
cell membrane
gp120
gp41
viral membrane
CD4 +
co-receptor
Pre-Hairpin
N
C
C peptides
slow rxn
Inhibited
Hairpin / Fusion
Post-Fusion

Figure 5

(A)


gp41 Clone Screen Data
% RNA Nitrocellulose Bound
45
40
35
30
25
20
15
10
5
0
D1
C1
G6
H3
F4
F6
A4
D2
G4
B4
H6
H2
E6
E4
A2
Positive Peptide
Negative Peptide
Mutant Peptide

(B)

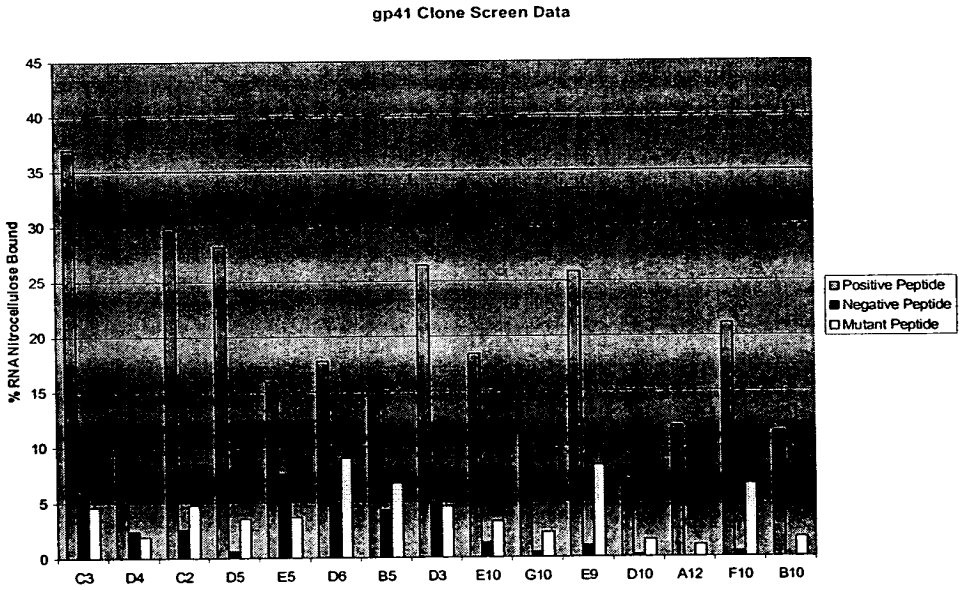

gp41 Clone Screen Data
% RNA Nitrocellulose Bound
45
40
35
30
25
20
15
10
5
0
C3
D4
C2
D5
E5
D6
B5
D3
E10
G10
E9
D10
A12
F10
B10
Positive Peptide
Negative Peptide
Mutant Peptide

Figure 6